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Histology of the Parotid Salivary Gland of the African Palm Squirrel (*Epixerus ebii*)

Histología de la Glándula Parótida Salival de la Ardilla de la Palma Africana (Epixerus ebii)

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Abstract

The parotid salivary gland microscopic morphology of the adult African Palm squirrel (*Epixerus ebii)* was investigated. This study was carried out to provide the basic histology of this organ in the palm squirrel, as there is dearth of information of its microscopic morphology from available literature. This becomes more important as the increasing use of this species of rodent as a pet and animal of importance in African game reserves for tourist attraction. The possibility of its domestication as a ready source of animal protein is also growing. Hence the need to understand the digestive biology to help animal nutritionist in feed formulation. The histology revealed the presence of only serous secretory acini cells. The serous cells were triangular to polyhedral shaped with a roundish basally located nucleus inside the mostly pinkish granulated cytoplasm. Myoepithelial cells were seen around the secretory cells and the intercalated ducts. The intralobular ducts of intercalated and striated ducts were lined by simple cuboidal and simple columnar cells, respectively. The excretory duct seen in the surrounding connective tissue was lined by stratified cuboidal cells. The granules in the cytoplasm of serous cells must be the digestive enzyme amylase that initiates

Resumen

Se investigó la histología de la glándula parótida salival de la ardilla adulta de la palma Africana (*Epixerus ebii)*. Este estudio se realizó para proporcionar la histología básica de este órgano en esta especie animal, ya que hay escasez de información relacionada con su morfología microscópica proveniente de la literatura disponible. Esto constituye un aspecto importante, por el incremento en el uso de esta especie de roedor como mascota y como animal de importancia en las reservas animales africanas de atracción turística. También está creciendo la posibilidad de su domesticación como una fuente rápida de proteína animal. De allí, la necesidad de entender la biología digestiva para ayudar al nutricionista en la formulación alimenticia. Los resultados de los estudios histológicos solamente revelaron la presencia de células acinares secretoras serosas. La forma de las células serosas varió de triangular a poliédrica, con un núcleo redondeado, localizado basalmente dentro de un citoplasma granulado predominantemente rosáceo. Se observaron células mioepiteliales alrededor de las células secretoras y de los ductos intercalados. Los ductos intralobulares de los ductos intercalados y estriados estaban alineados respectivamente en células cúbicas

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carbohydrate digestion. This well developed serous parotid salivary gland reflects the need for efficient carbohydrate digestion since the animal forages on fruits and nuts that are rich in carbohydrates. The granules could also contain lysozymes which serve in local defense against pathogenic agents in the oral cavity. This study for the first time documents the normal histology of the parotid salivary gland in this species, hence filling the knowledge gap that will help biologists to investigative more profundly. It will also help wild life veterinarians in diagnosis disease.

simples y en células columnares simples. El ducto excretor observado en el tejido conectivo circundante estaba alineado por células cúbicas estratificadas. Los gránulos en el citoplasma de las células serosas pudieran corresponderse con la enzima amilasa que inicia la digestión de carbohidratos. Esta glándula salival parótida bien desarrollada, refleja la necesidad de una eficiente digestión de carbohidratos, ya que el animal se alimenta de frutos y nueces ricos en estos principios inmediatos. Los gránulos también pudieran contener lisozimas que sirven para la defensa contra agentes patógenos presentes en la cavidad oral. Este estudio documenta por primera vez, la histología normal de la glándula parótida de esta especie, llenando por consiguiente, la brecha de conocimiento que ayudará a los biólogos a investigar más profundamente, y a los veterinarios que trabajan en fauna silvestre, en el diagnóstico de enfermedades.

(**Key words:** Animal histology, Salivary glands, African Palm, Squirrels, Serous Membrane, Nigeria)

(**Palabras clave**: Histología animal, Glándulas salivales, Palma africana, Ardilla, Membrana Serosa, Nigería)

INTRODUCTION

The parotid is one of the major mammalian salivary glands (Poddar and Jacob, 1977; Singh, 2003; Samuelson, 2007). The parotid glands parenchyma usually consist of two sections-the secretory acini cells and transport ducts (Martinez-Madrigal and Micheau, 1989; Sato and Miyoshi, 1998). The saliva secreted from the glands lubricates the mucosa of the oral cavity especially dry foods before swallowing (Vissink, 2010). Its high bicarbonate content has a buffering effect in the oral cavity. Its aqueous secretion provides a medium for dissolved food substances to stimulate the gustatory cells. It initiates carbohydrates digestion through the secretion of the enzyme amylase, and also controls bacterial flora by secreting lysozymes (Genkins, 1978). There are reports from literature that it secrets IgA, potassium and is involved in re-absorption of sodium (Ferraris *et al.*, 1999; Pijpe *et al.*, 2009).

Rodents are the largest order in mammals. They are used as pets, laboratory animals and sources of animal protein (Nzalak *et al.*, 2012). The squirrel being a rodent has wide geographical distribution, but the African palm squirrel is seen mostly in West Africa. From available literature, few studies have been conducted on the squirrel. These include reports on necropsy findings of nematodes on captive African squirrel (Craig *et al.*, 1998), rabies in fox squirrel (Cappucci *et al.*, 1972), hepatocellular carcinoma in Black-tailed prairie dogs- Cynomys ludivicionus (Garner *et al.*, 2004), natural infection of the ground squirrel with *Echinococcus granulosus* (Yang *et al.*, 2009). Whereas the morphology of the parotid salivary gland has been documented in the mouse (Jonick, 2001), histoenzymological detection of sialic acids in the Winster rat parotid salivary glands (Accili *et al.*, 1996), quantitative study of the parotid gland of the Fisher rat after orchiectomy (Jezek, *et al.*, 1999), even morphogenesis and functional differentiation of the rat parotid gland *in vivo* and *in vitro* (Lawson, 1970), but in the African palm squirrel, there is dearth of information on its basic biology from available literature, hence this microanatomic investigation on its parotid salivary gland. This documentation becomes important as the salivary glands of mammals are known to exhibit striking diversity in histology, ultrastructure and histochemistry (Pinkstaff, 1980). The result from this study will fill the knowledge gap that will aid further investigative research especially in studies on the role of myoepithelial cells in gland neoplasia. It will also help wild life biologist in understanding its adaptive digestive physiology and Veterinarians in managing the gland diseases of the species.

Materials and Methods

Five adult African palm squirrels (*Epixerus ebii)* of both sexes with an average weight of 163.5g and length of 18cm, captured in the wild from Olokoro Umuahia in Abia State, Nigeria from March to November 2012 using metal cage traps. Olokoro Umuahia is in the rainforest vegetation of southern Nigeria and is characterized by heavy rains and thick well grown mangrove forest trees. The squirrels were immediately transferred to the veterinary anatomy laboratory of Michael Okpara University of Agriculture, Umudike, for acclimatization. During this period, the animals were fed with grasses, oil palm fruit, and water *ad libitum*.

On the day of sacrifice, the squirrel was deep sedated with chloroform. The deep sedation was rapid, fast and painless for the animals. Death following deep sedation was rapid in the unconscious state.The weight of the animal was registred with a mettler balance (Model Ohaus Scout PRO-200) with a sensitivity of 0.1gm. Each squirrel was euthanized by a chloroform overdose and placed on dorsal recumbency. The animal was cut open through mid ventral incision from the inguinal region to the mandibular symphysis. The parotid salivary gland was dissected out from the lateral side of the base of the ear and slices fixed in 10% neutral buffered formalin. The tissues were passed through graded ethanol, cleared in xylene, impregnated and embedded in paraffin wax. Sections of 5 μm thick were obtained with a Leitz microtome, model 1512. They were stained with haematoxylin and eosin for light microscopy examination (Bancroft and Stevens, 1977). The slides were examined and photomicrographs taken with – Motican 2001 camera (Motican, UK) attached to an Olympus microscope.

Results

At high magnification, the parotid salivary gland was covered by a dense regular connective tissue capsule (Figure 1). Beneath this capsule, the lobulated gland contained serous cells in the secretory acini. The serous cells were triangular to polyhedral shaped with a roundish basally located nucleus inside the mostly pinkish granulated cytoplasm (Figure 1). Myoepithelial cells were seen surrounding the secretory acini cells and intercalated ducts (Figure 2). Each lobule contained intercalated ducts of simple cuboidal cells which were sandwiched between the secretory acini cells (Figures1, 2, 3), and larger striated or secretory ducts of simple columnar cells (Figure 3). Interlobular ducts of stratified cuboidal cells were seen as the excretory duct in the surrounding connective tissue fibres (Figure 4). Gland artery and vein were also seen in the surrounding connective tissue alongside the excretory duct (Figure 4).

Figure 1. Section of the parotid gland showing serous cells (SC), gland capsule (GC), of dense regular connective tissue and the intercalated ducts (DI) in each lobule. Note gland lobules separation L, within the covering capsule and connective tissue fibrocytes (black arrow). H&E x400

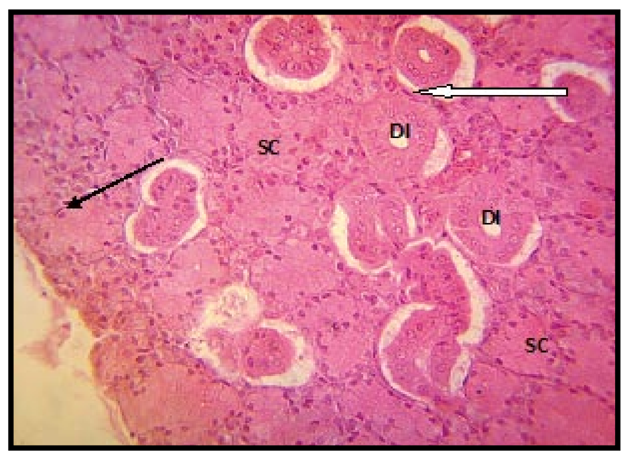

Figure 2. Section of the parotid salivary gland serous cells (SC), intercalated ducts (DI). Note myoepithelial cells (black arrow) surrounding the serous acini cells and intercalated ducts (white arrow). H&E x400

Discussion

This paper for the first time in available literature presents the histology of the African palm squirrel salivary gland. The covering capsule of dense regular connective tissue with fibrocytes is for protection of the serous secretory acini cells. A fibrous capsule of dense connective tissue has been also reported in the European hamster (*Cricetus cricetus*; Khojasteh and Delashoub, 2012). In this study, the parotid was seen to be composed of only serous cells. This observation of only serous cell has been reported in rabbits (EL-Ramli *et al.*, 2013), but a seromucous parotid gland has been reported in carnivores (Poddar and Jacob, 1977). The presence of well developed serous cells may be an adaptation for increased digestion of carbohydrates by amylase in the oral cavity. It may also be a need for increased production of the anti bacterial agent-lysozyme to help reduce rate of infection establishment in the wild (Ognean *et al.*, 2000).

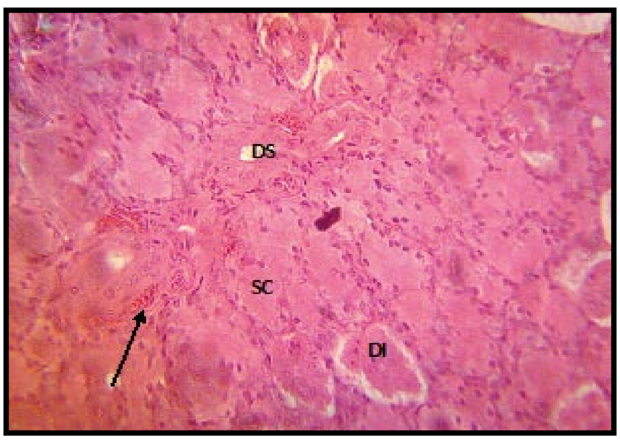

Figure 3. Section of the parotid salivary gland lobule showing serous cells (SC), intercalated ducts (DI), and striated duct (DS), and lobular capillary (black arrow). H&E x400

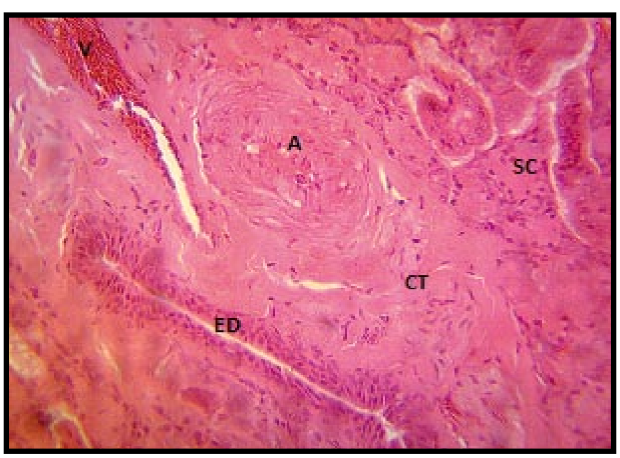

Figure 4. Section of the parotid salivary gland showing serous cells (SC). Note the excretory duct (ED), the large gland artery (A), and vein (V), in the surrounding connective tissue (CT). H&E x400

The intercalated duct of simple cuboidal epithelium functions to transport secretions from the acini cells to the striated duct. This simple cuboidal epithelium in the intercalated duct has been reported in other rodents (Amano *et al.*, 2012). In the Fisher rat the intercalated duct was lined by simple squamous to cuboidal cells with an elongated or oval nucleus (Jezek *et al.*, 1999). The striated duct of simple columnar epithelium transports secretions from the intercalated duct to the excretory duct. A tall cuboidal epithelium in the striated duct has been reported in the submandibular gland of gerbil (*Meriones unguiculatus*; Bazan *et al.*, 2001) but in the Fisher rat the striated duct was lined by cuboidal to columnar epithelium (Jezek, *et al.*, 1999). The intercalated and striated ducts are referred to as intralobular duct, and this has been reported in other rodents (Amano *et al*, 2012). In this study, the presence of a large number of intercalated ducts in the parotid gland with only serous cells may be a functional morphological specialization for increased transport of digestive enzyme amylase from the secretory acini into the striated duct, thus increasing the rate of digestion of carbohydrates by these enzymes as more serious fluid is transported per unit of time into the oral cavity. The excretory duct of stratified cuboidal epithelium in the interlobular duct finally delivers the products of the gland into the oral cavity. The presence of stratified epithelium in the excretory duct may reflect the need for protection of underlying basement membrane by occasional action of activated serous fluid enzymes.

The myoepithelial cells surrounding the secretory acini cells and intercalated ducts provides contractile force to help expel this secretion from the acini cells and push them through the intercalated duct (Redman, 1994; Amano *et al.*, 2012). This contraction of the myoepithelial cells is under the control of autonomic nervous stimulation (Ogawa, 2003). There is a report on the ability of the myoepithelial cells to store glycogen (Batsakis *et al.*, 1983), but this was not demonstrated in this study. The absence of myoepithelial cells in rat parotid salivary gland and their occasional presence in human salivary gland have been reported (Ogawa, 2003). These myoepithelial cells have been incriminated in the pathogenesis of salivary gland tumours in humans (Batsakis *et al.*, 1983; Martinez-Madrigal and Micheau, 1989).

Conclusion

The micromorphology of the African palm squirrel parotid salivary gland from this study revealed a serous gland. The well developed serous acini cells is a functional adaptation for increased rate of digestion by salivary gland enzymes in the oral cavity as the animal is a common prey, hence needs this increased rate as the luxury of preydator free environment is lacking. The well developed parotid salivary gland from this study would serve as a model for other biomedical researches like the amylase rate of secretion, digestive zymogen activities and myoepithelial cells functions.

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